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Observations on Morphology and the Response of Hyphae to Temperature by Australian and French Isolates of *Lepista*

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Abstract

The development of viable commercial cultivation techniques for the edible fungus *Lepista nuda* (Bull.: Fr.) Cooke is necessary to expand the range of species available to the exotic/wild mushroom growing industry in Australia. This study provides new data on the effect of temperature on hyphal growth of Australian isolates of *Lepista*. Wild *Lepista* were collected from selected cool (4–25°C during growing season) and warm (8–35°C during growing season) climatic regions in Australia and pure vegetative cultures isolated. These collections differed morphologically from overseas isolates but whether these are new species or varieties or the result of environment and habitat has yet to be determined. The optimum temperature parameters for hyphal growth were determined and comparisons made with French cool (average day temperature 11°C) and warm (average day temperature 14°C) climatic region isolates. The range of temperatures for the mycelial growth of Australian and French isolates *in vitro* was 5–35°C and 5–30°C respectively. The growth rate of Australian isolates was more than double that of French isolates at the optimum temperatures.

Introduction

There are 20–25 species of fungi suitable for amateur or extensive small scale commercial production and four species suitable for intensive commercial production (Olivier 1991). In Australia, amongst the gilled fungi, there are a number of edible species in the families Agaricaceae, Bolbitiaceae and Tricholomataceae. The mushroom *Lepista nuda* (Bull. : Fr.) Cooke (= *Tricholoma nudum* (Bull. : Fr.) P. Kumm.; *Rhodopaxillus nudus* (Bull. : Fr.) Maire; *Clitocybe nuda* (Bull. : Fr.) H.E. Bigelow & A.H. Sm.), of the family Tricholomataceae, commonly known as Wood Blewitt, is well known in Europe and Britain where it has an international gastronomic reputation (Krieger 1967, Dickinson and Lucas 1979, Jordan 1989). It has a slightly aromatic smell and sweetish taste (Jordan 1995) and is better cooked, fried or baked, than eaten raw (Dickinson and Lucas 1979, Jordan 1989). *Lepista nuda* deserves research into methods of cultivation because of its eating characteristics and the need for new wild/exotic species of fungi by the Australian mushroom industry.

A study of the morphology and growing characteristics of Australian *Lepista nuda* is required to select isolates attractive for consumption and commercial production. The optimum temperature for hyphal growth is an important factor for colonisation of substrate and shorter cropping times.

Materials and methods

Isolates

Basidiocarps were collected from cool and warm climatic regions in eastern Australia and their genera and species identified. Axenic cultures were then prepared from the basidiocarp. These isolates were identified as *Lepista nuda* and then further groupings were determined on the basis of macroscopic and microscopic characteristics. Isolates from different groups, habitats and environments were then selected for further study into the response of hyphae to temperature. Vegetative cultures of *Lepista nuda* from cool and warm climate regions of France were supplied by the Centre de Champignons, Institut National Research Agronomique, Bordeaux.

Temperature

A 4 mm disc of hyphae was cut from the growing edge of an 8 day old culture and placed with the hyphal surface in contact with the agar at the centre of a petri dish containing malt extract agar plus 2% yeast extract (MEAY). Five replicates of each isolate were placed at ten temperatures ranging from 5–40°C and incubated in the dark for 8 days. Radial growth was measured between the outer edge of the colony and the inoculum along two axes at 90° to each other. This figure was averaged to determine the radial growth of hyphae from the edge of the inoculum.

Results

Isolates

Australian isolates were collected from a variety of habitats: leaf litter under shrubs (*Rhododendron* and *Camellia*); leaf litter under trees (*Cedrus deodara* and *Quercus suber*); and on open grassland (*Poa pratensis*, *Pennisetum* sp. and *Cynodon dactylon*).

All basidiocarps of the Australian isolates were either violet, lilac or bluish overall and all were assigned to the section *Genuinae* Konrad and Maublanc. The spore colour of all isolates was salmon pink, the shape ellipsoid and the surface minutely warty. Using the dichotomous keys of Pegler (1977), Moser (1978) and Breitenbach and Kranzlin (1991) and descriptions from Guinberteau *et al.* (1989) isolates were keyed out to the species *Lepista nuda*, with four subgroups identified (Table 1) using macroscopic characteristics. These may be taxonomic subgroups but they are also useful for identification of isolates attractive to the market place.

Temperature

The optimum temperature for hyphal growth of the Australian and French isolates was found to be 25–30°C and 20–24°C respectively with no growth at 40°C. Significant differences (using ANOVA) were found in the radial growth of hyphae between Australian and French isolates (Table 2).

Discussion

Species of *Lepista* are widely distributed in different ecosystems throughout the world. *Lepista nuda* has been collected in Switzerland, Britain and France in late summer-autumn, rarely spring (Breitenbach and Kranzlin 1991); autumn to early winter (Phillips 1985); and late autumn-winter (Guinberteau *et al.* 1989). The growth habit of this genus is variable being found in groups, rings and caespitose, but it is rarely found

Table 1. Subgroups of Australian *Lepista nuda*, on the basis of macroscopic characteristics

Characteristic		Sub group A	B	C	D
Basidiocarp	shape and form	robust and fleshy	less robust than A but fleshy	bends easily having stipe: pileus ratio smaller than B	similar to form B but often with umbo
	height (mm)	70–110	70	95	75
Pileus	colour	lilaceous brown and shiny	lilac to lilaceous brown and shiny	lilac	bright or deep lilac or lilaceous brown
	diameter (mm)	65–132	35–40	75	50–75
	shape	convex to shallow convex with age	shallow convex	convex to plane	shallow convex or plane and with/without umbo
Stipe	colour	lilac to lilac purple, darker at base	lilac	lilac	lilac to lilaceous brown
	size (mm)	50–78 x 25–33	60 x 11	75 x 8	45–65 x 7–11
Lamellae colour		lilac purple to deep lilac	lilac	lilac	lilac or lilaceous pink or deep lilac
Habitat		leaf litter under *Rhododendron*, *Camellia*, *Cedrus deodara*, *Quercus suber*	lawns — *Poa pratensis*	pasture — *Pennisetum*	roadsides, pasture, gardens — *Pennisetum*, *Cynodon dactylon*
Habit	group	ring	group	group	ring or group
Climate[1]		cool	cool	warm	warm

Table 2. Hyphal growth of Australian and French isolates of *Lepista nuda* in response to temperature

Group[1]	Optimum temperature (°C)[2]	Radial growth of hyphae (mm)[2]
A	25	17.98
B	25	24.59
C	30	24.68
D	25	29.97
FW	24	11.38
FC	20	12.46

[1]Groups A,B,C,D are Australian subgroup isolates with A and B being cool climate and C and D warm climate; FW are French warm climate isolates; FC are French cool climate isolates.
[2]Respresentative mean of all isolates within the subgroup.

solitary. It grows in coniferous and deciduous woods, pasture, roadsides, gardens, compost heaps and under shrubs. The habitats and season of growth of Australian isolates appear similar to that of the European ones. Australian isolates differ morphologically from overseas isolates but whether they are new species, varieties or the result of environmental differences and habitat has yet to be determined. Therefore an unequivocal resolution of isolates was not possible but four sub groups were identified.

The optimum growing temperature for Australian and French isolates growing on MEAY are 30 and 25°C respectively. In addition the hyphal growth rates of Australian isolates are almost double that of French for both warm and cool isolates. This has significant implications on the rate of growth of hyphae through substrate and it is expected to lead to reduced time for colonisation of substrate and a shorter growing period. The maximum temperatures tolerated by Australian isolates are higher than French isolates at 35°C and 30°C respectively with Australian isolates having less growth at 5°C than French isolates. This may indicate that the process of basidiocarp induction could be encouraged to occur at higher temperatures than are currently accepted in Europe. Research into this next phase of commercialisation is currently being undertaken.

The four Australian subgroups could be the result of nutrition, age and/or weather. Alternatively, the combination of morphological and temperature response data may indicate that the Australian isolates are new strains, varieties or species. This information on morphology and effect of temperature on hyphal growth of Australian *Lepista* isolates will form the basis for the development of a viable commercial cultivation technique for this edible fungus.

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References

Breitenbach, J., and Kranzlin, F. (1991). 'Fungi of Switzerland Volume 3 — Boletes and Agarics, 1st part.' (Edition Mykologia: Lucerne, Switzerland.)

Dickinson, C., and Lucas, J. (1979). 'The Encyclopedia of Mushrooms.' (G.P. Putnam's Sons: New York.)

Guinberteau, J., Olivier, J.M., and Bordaberry, M.R. (1989). Donnees recentes sur la culture des 'Pieds bleus' (*Lepista* sp.). *PHM Revue Horticole* **298**, 17–22.

Jordan, M. (1989). 'Mushroom Magic.' (Elm Tree Books: United Kingdom.)

Jordan, M. (1995). 'The Ultimate Mushroom Book.' (Annes Publishing Limited: London.)

Krieger, L.C.C. (1967). 'The Mushroom Handbook.' (Dover Publications, Inc.: New York.)

Moser, M. (1978). 'Keys to Agarics and Boleti (Polyporales, Boletales, Agaricales, Russulales).' (Roger Phillips: London.)

Olivier, J.M. (1991). Champignon comestibles et diversification. Mushroom Information **4**, 20–1.

Pegler, D.N. (1977). A Preliminary Agaric Flora of East Africa. *Kew Bulletin Additional Series* **6**, 1–615.

Phillips, R. (1985). 'Mushrooms and Other Fungi of Great Britain and Europe.' (Ward Lock Ltd.: London.)

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